

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5745	glucoamylase\$1	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:31
L2	353	EMERSONII OR TALAROMYCES	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:32
L3	12160	thermostab\$	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:32
L4	31	1 same 2	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:32
L5	44	1 near3 3	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:33
L6	124420	(ACTIVIT\$5 NEAR4 (INCREAS\$ OR HIGH\$5))	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:33
L7	65	1 near5 6	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:33
L8	23	3 and 7	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:34
L9	44	4 or 8	US-PGPUB; USPAT	OR	OFF	2005/01/07 17:34

PGPUB-DOCUMENT-NUMBER: 20040253696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040253696 A1

TITLE: Fermentation processes and compositions

PUBLICATION-DATE: December 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Grichko, Varvara	Raleigh	NC	US	

APPL-NO: 10/ 459315

DATE FILED: June 10, 2003

US-CL-CURRENT: 435/161

ABSTRACT:

The present invention provides improved fermentation processes, including for use in an ethanol production process. The improved fermentation processes include applying at least one fatty acid oxidizing enzyme (such as a lipoxygenase) in a fermentation process. The improved fermentation process may also involve the addition of various additional enzymes and growth stimulators for the fermenting microorganisms, including vitamins and mineral.

----- KWIC -----

Detail Description Paragraph - DETX (83):

[0096] Other *Aspergillus glucoamylase* variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus *Clostridium*, in particular *C. thermoamylolyticum* (EP 135,138), and *C. thermohydrosulfuricum* (WO 86/01831).

PGPUB-DOCUMENT-NUMBER: 20040229367

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040229367 A1

TITLE: Methods for monitoring multiple gene expression

PUBLICATION-DATE: November 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Berka, Randy M.	Davis	CA	US	
Rey, Michael W.	Davis	CA	US	
Shuster, Jeffrey R.	Davis	CA	US	
Kauppinen, Sakari	Smoerum		DK	
Clausen, Ib Groth	Hillerod		DK	
Olsen, Peter Bjarke	Copenhagen		DK	

APPL-NO: 10/ 653047

DATE FILED: August 29, 2003

RELATED-US-APPL-DATA:

child 10653047 A1 20030829

parent division-of 09533559 20000322 US PENDING

child 09533559 20000322 US

parent continuation-in-part-of 09273623 19990322 US ABANDONED

US-CL-CURRENT: 435/484, 435/254.3

ABSTRACT:

The present invention relates to methods for monitoring differential expression of a plurality of genes in a first filamentous fungal cell relative to expression of the same genes in one or more second filamentous fungal cells using microarrays containing filamentous fungal expressed sequenced tags. The present invention also relates to filamentous fungal expressed sequenced tags and to computer readable media and substrates containing such expressed sequenced tags for monitoring expression of a plurality of genes in filamentous fungal cells.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a divisional of pending U.S. application Ser. No. 09/533,559 filed Mar. 22, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/273,623 filed Mar. 22, 1999, now abandoned, which applications are fully incorporated herein by reference.

----- KWIC -----

Detail Description Table CWU - DETL (1):

1TABLE 1 Fusarium venenatum ESTs Sequence Functional Listing zscore

Annotation Database category 1 2667.2 Talaromyces emersonii geneseq Y23339 ND  
glucoamylase 2 4203.8 ELONGATION FACTOR 2 swissprot P32324 ND (EF-2). 3  
 3198.0 ATP SYNTHASE BETA swissnew P23704 ND CHAIN, MITOCHONDRIAL PRECURSOR  
 (EC 3.6.1.34). 4 1956.9 AMMONIUM sptrembl q9y877 Inorganic ion TRANSPORTER  
 MEPA transport and metabolism 6 2960.4 ELONGATION FACTOR 1- swissprot  
 P34825 ND ALPHA (EF-1-ALPHA). 7 2917.2 ABC1 TRANSPORTER. sptrembl O13407 ND  
 8 2791.3 GAMMA-ACTIN. tremblnew ND AAF00008 9 2703.6 TUBULIN BETA CHAIN.  
 swissprot P53374 ND 12 2561.0 CITRATE SYNTHASE, swissprot P34085 ND  
 MITOCHONDRIAL PRECURSOR (EC 4.1.3.7). 13 2554.9 60S RIBOSOMAL PROTEIN  
 tremblnew ND L3. AAF15600 14 2522.1 Microscilla furvescens geneseq  
 Inorganic ion catalase-53CA1. W33810 transport and metabolism. 15 2436.2  
 Cladosporium herbarum geneseq R71891 Energy allergen Clah53. production and  
 conversion 16 2350.6 THIAZOLE BIOSYNTHETIC swissprot P23618 ND ENZYME  
 PRECURSOR (STRESS-INDUCIBLE PROTEIN ST135). 17 2331.8 SUBTILISIN-LIKE  
 tremblnew Posttranslational PROTEASE PR1H. CAB63907 modification, protein  
 turnover, chaperones 18 2293.3 ALPHA-TUBULIN. tremblnew ND CAA74848 21  
 2165.4 GUANINE NUCLEOTIDE- swissprot Q01369 ND BINDING PROTEIN BETA  
 SUBUNIT-LIKE PROTEIN (CROSS-PATHWAY CONTROL WD-REPEAT PROTEIN CPC-2). 22  
 2148.3 AMINO-ACID PERMEASE swissprot P34054 ND INDA1. 24 2125.9 NMT1 PROTEIN  
 swissprot P42882 Inorganic ion HOMOLOG. transport and metabolism 25 2090.9  
 PUTATIVE MULTICOPPER swissprot P43561 ND OXIDASE YFL041W PRECURSOR (EC  
 1.-.-). 26 2082.1 PLASMA MEMBRANE swissprot Q07421 Inorganic ion ATPASE (EC  
 3.6.1.35) transport and (PROTON PUMP). metabolism 27 2071.7 PLASMA MEMBRANE  
 swissprot Q07421 ND ATPASE (EC 3.6.1.35) (PROTON PUMP). 28 2039.0 ADP, ATP  
 CARRIER swissprot P02723 ND PROTEIN (ADP/ATP TRANSLOCASE) (ADENINE  
 NUCLEOTIDE TRANSLOCATOR) (ANT). 29 2026.4 ATP SYNTHASE ALPHA swissnew P37211  
 ND CHAIN, MITOCHONDRIAL PRECURSOR (EC 3.6.1.34). 30 2025.5 HEAT SHOCK 70 KD  
 swissprot Q05944 Posttranslational PROTEIN. modification, protein turnover,  
 chaperones 31 1960.7 T. harzianum exochitinase. geneseq ND W01639 32 1916.8  
 PUTATIVE DIHYDROXY- swissprot Q10318 ND ACID DEHYDRATASE, MITOCHONDRIAL  
 PRECURSOR (EC 4.2.1.9) (DAD) (2,3-DIHYDROXY ACID HYDROLYASE). 33 1905.0  
 CUTINASE swissprot P52958 ND TRANSCRIPTION FACTOR 1 ALPHA. 34 1903.2  
 EUKARYOTIC INITIATION swissprot Q10055 ND FACTOR 4A-LIKE PROTEIN C1F5.10. 35  
 1894.8 NADH DEHYDROGENASE sptrembl Q01388 ND SUBUNIT. 36 1869.1 TRANSLATION  
 RELEASE sptrembl 042787 Amino acid FACTOR ERF3. transport and metabolism 37  
 1868.4 GLYCERALDEHYDE 3- swissprot P35143 ND PHOSPHATE DEHYDROGENASE (EC  
 1.2.1.12) (GAPDH). 38 1852.7 VACUOLAR ATP swissprot P11592 ND SYNTHASE  
 CATALYTIC SUBUNIT A (EC 3.6.1.34) (V- ATPASE 67 KD SUBUNIT). 39 1838.0  
 PEROXISOMAL swissnew Q01373 ND HYDRATASE- DEHYDROGENASE- EPIMERASE (HDE)  
 (MULTIFUNCTIONAL BETA-OXIDATION PROTEIN) (MFP) [INCLUDES: 2-ENOYL-COA  
 HYDRATASE (BC 4.2.1.-); D- 3-HYDROXYACYL COA DEHYDROGENASE (BC 1.1.1.-)].  
 42 1816.8 N. crassa glucoamylase. geneseq R71034 ND 43 1798.7 XANTHINE  
 swissprot Q12553 ND DEHYDROGENASE (EC 1.1.1.204) (PURINE HYDROXYLASE I). 44  
 1769.7 78 KD GLUCOSE- swissnew P78695 ND REGULATED PROTEIN HOMOLOG  
 PRECURSOR (GRP 78) (IMMUNOGLOBULIN HEAVY CHAIN BINDING PROTEIN HOMOLOG)  
 (BIP). 45 1769.5 RIBONUCLEOSIDE- swissprot P31350 Nucleotide DIPHOSPHATE  
 REDUCTASE transport M2 CHAIN (EC 1.17.4.1) (RIBONUCLEOTIDE REDUCTASE). 47  
 1740.5 6-PHOSPHOGLUCONATE swissprot P38720 ND DEHYDROGENASE, DECARBOXYLATING  
 1 (EC 1.1.1.44). 48 1711.5 SERINE/THREONINE swissprot P48580 ND PROTEIN  
 PHOSPHATASE PP2A CATALYTIC SUBUNIT (EC 3.1.3.16). 49 1701.5 GEL1 PROTEIN.  
 sptrembl O74687 ND 50 1691.1 PUTATIVE LYSYL-TRNA tremblnew ND SYNTHETASE.  
 CABS2801 51 1671.7 SILIMAR TO GLUTAMATE sptrembl Q05567 ND DECARBOXYLASE.  
 52 1634.0 GLYCOGEN SYNTHASE. sptrembl O93869 Cell envelope biogenesis, outer  
 membrane 53 1630.0 CHROMOSOME XVI sptrembl Q12464 DNA replication, READING  
 FRAME ORF recombination YPL235W. and repair 54 1626.3 TRANSALDOLASE (EC  
 sptrembl O42700 Carbohydrate 2.2.1.2). transport and metabolism 56 1614.8  
 KETOL-ACID swissnew P38674 Amino acid REDUCTOISOMERASE transport and  
 PRECURSOR (EC 1.1.1.86) metabolism (ACETOHYDROXY-ACID REDUCTOISOMERASE)  
 (ALPHA-KETO-BETA- HYDROXYLACIL REDUCTOISOMERASE). 57 1609.5 GLUTAMATE

SYNTHASE swissnew Q03460 ND [NADH] PRECURSOR (EC 1.4.1.14) (NADH-GOGAT). 58  
 1600.3 DICARBOXYLIC AMINO swissprot P53388 ND ACID PERMEASE. 59 1599.3 Yeast  
 ribosomal protein S7. geneseq ND W36115 60 1579.6 SODIUM TRANSPORT sptrembl  
 Q00877 ND ATPASE FST. 61 1577.3 SIMILAR TO ASPARTATE sptrembl Q17994 Amino  
 acid AMINOTRANSFERASE. transport and metabolism 63 1562.2 EUKARYOTIC  
 INITIATION swissprot P47943 ND FACTOR 4A (EIF-4A). 65 1552.1 SUCCINATE  
 swissnew O42772 ND DEHYDROGENASE [UBIQUINONE] IRON- SULFUR PROTEIN,  
 MITOCHONDRIAL PRECURSOR (EC 1.3.5.1) (IP). 67 1546.9 ACTIN-LIKE PROTEIN 3.  
 swissprot P78712 Cell division and chromosome partitioning 68 1538.6  
 HYPOTHETICAL 44.3 KD sptrembl O13998 ND PROTEIN C27E2.03C IN CHROMOSOME I.  
 69 1529.6 BETA-GLUCOSIDASE 1 swissprot P48825 ND PRECURSOR (EC 3.2.1.21)  
 (GENTIOBIASE) (CELLOBIASE) (BETA-D- GLUCOSIDE GLUCOHYDROLASE). 70 1528.3  
 GLUCOSE-6-PHOSPHATE swissprot P12709 Carbohydrate ISOMERASE (GPI) (EC  
 transport and 5.3.1.9) (PHOSPHOGLUCOSE metabolism ISOMERASE) (PGI)  
 (PHOSPHOHEXOSE ISOMERASE) (PHI). 71 1527.0 2-OXOGLUTARATE swissprot P20967  
 ND DEHYDROGENASE E1 COMPONENT, MITOCHONDRIAL PRECURSOR (EC 1.2.4.2)  
 (ALPHA-KETOGLUTARATE DEHYDROGENASE). 72 1505.5 PROTEIN DISULPHIDE sptrembl  
 O74568 ND

PGPUB-DOCUMENT-NUMBER: 20040219649

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040219649 A1

TITLE: Alcohol product processes

PUBLICATION-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Olsen, Hans Sejr	Holte		DK	
Pedersen, Sven	Gentofte		DK	
Festersen, Rikke Monica	Copenhagen K		DK	

APPL-NO: 10/ 797393

DATE FILED: March 10, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60453326 20030310 US

US-CL-CURRENT: 435/161, 435/170 , 435/201

ABSTRACT:

The present invention relates to processes for production of an alcohol product from granular starch comprising a pre-treatment at an elevated temperature below the initial gelatinization temperature of said granular starch followed by simultaneous saccharification and fermentation, and optionally recovery of ethanol.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims, under 35 U.S.C. 119, the benefit of U.S. provisional application No. 60/453,326 filed on March 10, 2003 the contents of which are fully incorporated herein by reference.

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Detail Description Paragraph - DETX (12):

[0026] Other preferred glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32, 153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215), Clostridium, in particular C. thermoamylolyticum (EP135,138), and C. thermohydrosulfuricum (WO86/01831).

Claims Text - CLTX (17):

53. The process according to claim 1, wherein the glucoamylase is obtained from a strain of Aspergillus, Talaromyces or Clostridium.

PGPUB-DOCUMENT-NUMBER: 20040142434

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040142434 A1

TITLE: Thermostable glucoamylase

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nielsen, Bjarne Ronfeldt	Virum		DK	
Nielsen, Ruby Illum	Farum		DK	
Lehmbeck, Jan	Vekso		DK	

APPL-NO: 10/ 625115

DATE FILED: July 22, 2003

RELATED-US-APPL-DATA:

child 10625115 A1 20030722

parent division-of 09821616 20010329 US GRANTED

parent-patent 6620924 US

child 09821616 20010329 US

parent continuation-of 09199290 19981124 US GRANTED

parent-patent 6255084 US

child 09199290 19981124 US

parent continuation-in-part-of 08979673 19971126 US ABANDONED

child 09199290 19981124 US

parent continuation-in-part-of 09107657 19980630 US ABANDONED

non-provisional-of-provisional 60070746 19980108 US

non-provisional-of-provisional 60094344 19980728 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1557/97	1997DK-1557/97	December 30, 1997
DK	1998 00925	1998DK-1998 00925	July 10, 1998

US-CL-CURRENT: 435/105, 435/203

ABSTRACT:

The invention relates to starch conversion processes using glucoamylases derived from Talaromyces emersonii and related glucoamylases.

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of application Ser. No. 09/821,616, filed Mar. 29, 2001, which is a continuation of application Ser. No. 09/199,290 filed Nov. 24, 1998, which is a continuation-in-part of application Ser. Nos. 08/979,673 and 09/107,657 filed Nov. 26, 1997 and Jun. 30, 1998, respectively, and claims priority under 35 U.S.C. 119 of Danish application nos. 1557/97 and PA 1998 00925 filed Dec. 30, 1997 and Jul. 10, 1998, respectively, and U.S. application Nos. 60/070,746 and 60/094,344 filed Jan. 8, 1998 and Jul. 28, 1998, respectively, the contents of which are fully incorporated herein by reference.

----- KWIC -----

### Abstract Paragraph - ABTX (1):

The invention relates to starch conversion processes using glucoamylases derived from Talaromyces emersonii and related glucoamylases.

### Title - TTL (1):

Thermostable glucoamylase

### Summary of Invention Paragraph - BSTX (2):

[0002] The present invention relates to a thermostable glucoamylase suitable for, e.g., starch conversion, e.g., for producing glucose from starch. The present invention also relates to the use of said thermostable glucoamylase in various processes, in particular in the saccharification step in starch conversion processes.

### Summary of Invention Paragraph - BSTX (8):

[0007] U.S. Pat. No. 4,247,637 describes a thermostable glucoamylase having a molecular weight of about 31,000 Da derived from Talaromyces duponti suitable for saccharifying a liquefied starch solution to a syrup. The glucoamylase is stated to retain at least about 90% of its initial glucoamylase activity when held at 70.degree. C. for 10 minutes at pH 4.5.

### Summary of Invention Paragraph - BSTX (9):

[0008] U.S. Pat. No. 4,587,215 discloses a thermostable amyloglucosidase derived from the species Talaromyces thermophilus with a molecular weight of about 45,000 Da. The disclosed amyloglucosidase (or glucoamylase) loses its enzymatic activity in two distinct phases, an initial period of rapid decay followed by a period of slow decay. At 70.degree. C. (pH=5.0) the half-life for the fast decay is about 18 minutes with no measurable loss of activity within an hour in the second phase of decay.

### Summary of Invention Paragraph - BSTX (12):

[0010] The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use, e.g., in the saccharification step in starch conversion processes.

### Summary of Invention Paragraph - BSTX (15):

[0013] The inventors of the present invention have isolated, purified and characterized a thermostable glucoamylase from a strain of Talaromyces emersonii now deposited with the Centraalbureau voor Schimmelcultures under the number CBS 793.97.

### Summary of Invention Paragraph - BSTX (20):

[0018] The isolated glucoamylase has a very high thermal stability in comparison to prior art glucoamylases, such as the *Aspergillus niger*



glucoamylase (available from Novo Nordisk ANS under the trade name AMG). The T.sub.1/2 (half-life) was determined to be about 120 minutes at 70.degree. C. (pH 4.5) as described in Example 2 below. The T.sub.1/2 of the recombinant T. emersonii AMG expressed in yeast was determined to be about 110 minutes as described in Example 12.

Summary of Invention Paragraph - BSTX (29):

[0027] Finally, the invention relates to an isolated pure culture of the microorganism Talaromyces emersonii CBS 793.97 or a mutant thereof capable of producing a glucoamylase of the invention.

Brief Description of Drawings Paragraph - DRTX

(2):

[0028] FIG. 1 shows the SDS-PAGE gel (stained with Coomassie Blue) used for determining the molecular weight (M.sub.w) of the purified Talaromyces emersonii CBS 793.97 glucoamylase of the present invention.

Brief Description of Drawings Paragraph - DRTX

(6):

[0032] FIG. 2 shows the pH activity profile of Talaromyces emersonii and Aspergillus niger glucoamylase (AMG) in 0.5% maltose at 60.degree. C.;

Brief Description of Drawings Paragraph - DRTX

(7):

[0033] FIG. 3 shows the temperature activity profile of the Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG)

Brief Description of Drawings Paragraph - DRTX

(8):

[0034] FIG. 4 shows the curve for determining T.sub.1/2 (half-life) in 50 mM NaOAc, 0.2 AGU/ml, pH 4.5, at 70.degree. C. of Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG);

Brief Description of Drawings Paragraph - DRTX

(15):

[0041] FIG. 10 shows the SDS page gel of two transformants, JaL228#5.77 and HowB112#8.10, expressing the Talaromyces emersonii glucoamylase of the invention. JaL228 and HowB112 are the untransformed parent strains. MW: Promega's Protein Molecular;

Brief Description of Drawings Paragraph - DRTX

(18):

[0044] FIG. 13 shows the result of the test for determining the thermostability of recombinant Talaromyces emersonii AMG produced in yeast at 70.degree. C., pH 4.5, 0.2 AGU/ml. T.sub.1/2 was determined to about 110.degree. C.

Detail Description Paragraph - DETX (2):

[0045] The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use in, e.g., the saccharification step in a starch conversion process.

Detail Description Paragraph - DETX (3):

[0046] The inventors of the present invention have isolated, purified and characterized a glucoamylase from a strain of Talaromyces emersonii CBS 793.97. The glucoamylase turned out to have a very high thermal stability in comparison to prior art glucoamylases.

Detail Description Paragraph - DETX (5):

[0048] T.sub.1/2 (half-life) of the isolated Talaromyces emersonii CBS 793.97 glucoamylase was determined to be about 120 minutes at 70.degree. C. as described in Example 2 below and to be about 110.degree. C. for the T. emersonii produced in yeast as described in Example 12.

Detail Description Paragraph - DETX (15):

[0058] Talaromyces Emersonii Glucoamylase Amino Acid Sequence

Detail Description Paragraph - DETX (16):

[0059] The inventors have sequenced the thermostable glucoamylase derived from Talaromyces emersonii CBS 793.97 as will be described further in the Example 3 below. According to the invention the Talaromyces AMG may have a Asp145Asn (or D145N) substitution (using SEQ ID NO: 7 numbering).

Detail Description Paragraph - DETX (37):

[0080] The present invention provides a method of using the thermostable glucoamylase of the invention for producing glucose and the like from starch. Generally, the method includes the steps of partially hydrolyzing precursor starch in the presence of .alpha.-amylase and then further hydrolyzing the release of D-glucose from the non-reducing ends of the starch or related oligo- and polysaccharide molecules in the presence of glucoamylase by cleaving .alpha.-(14) and .alpha.-(16) glucosidic bonds.

Detail Description Paragraph - DETX (40):

[0083] By using a thermostable glucoamylase of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This applies both for traditional batch processes (described above) and for continuous saccharification processes.

Detail Description Paragraph - DETX (41):

[0084] Actually, continuous saccharification processes including one or more membrane separation steps, i.e., filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane. Therefore, a thermostable glucoamylase of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price within a period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detail Description Paragraph - DETX (42):

[0085] The activity of a glucoamylase of the invention is generally substantially higher at temperatures between 60.degree. C.-80.degree. C. than at the traditionally used temperature between 30-60.degree. C. Therefore, by increasing the temperature at which the glucoamylase operates the saccharification process may be carried out within a shorter period of time or the process may be carried out using lower enzyme dosage.

Detail Description Paragraph - DETX (44):

[0087] By using a glucoamylase with increased specific activity (measured as activity towards maltose), a lower enzyme dosage may be required in the saccharification process.

Detail Description Paragraph - DETX (55):

[0098] Glucoamylase derived from the deposited filamentous fungus Talaromyces emersonii CBS No. 793.97 has been deposited with the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands, for the

purposes of patent procedure on the date indicated below. CBS being an international depository under the Budapest Treaty affords permanence of the deposit in accordance with rule 9 of said treaty.

Detail Description Paragraph - DETX (66):

[0109] T. emersonii glucoamylase gene with introns is shown in FIG. 5 and SEQ ID NO: 33. The introns are shown in FIG. 5.

Detail Description Paragraph - DETX (128):

[0168] Characterisation of the Talaromyces Emersonii Glucoamylase

Detail Description Paragraph - DETX (129):

[0169] The purified Talaromyces emersonii CBS 793.97 glucoamylase was used for characterisation.

Detail Description Paragraph - DETX (135):

[0175] The pH-activity dependency of the Talaromyces emersonii glucoamylase was determined and compared with profile of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (138):

[0178] The temperature-activity dependency of the Talaromyces emersonii glucoamylase of the invention was determined and compared with the profile of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (141):

[0181] The thermal stability of the Talaromyces emersonii glucoamylase was determined and compared with the thermal stability of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (143):

[0183] The T1/2 of the Talaromyces emersonii glucoamylase was determined to about 120 minutes at 70.degree. C. The T1/2 of the Aspergillus niger glucoamylase was determined to 7 minutes under the same conditions (See FIG. 4).

Detail Description Paragraph - DETX (148):

[0187] Sequencing of the N-terminal of T. Emersonii Glucoamylase

Detail Description Paragraph - DETX (149):

[0188] The N-terminal amino acid sequence of T. emersonii glucoamylase was determined following SDS-PAGE and electroblotting onto a PVDF-membrane. Peptides were derived from reduced and S-carboxymethylated glucoamylase by cleaving with a lysyl-specific protease. The resulting peptides were fractionated and re-purified using RP-HPLC before subjected to N-terminal sequence determination.

Detail Description Paragraph - DETX (151):

[0189] The Full Length T. Emersonii Glucoamylase

Detail Description Paragraph - DETX (152):

[0190] The full length T. emersonii glucoamylase amino acid sequence shown in SEQ ID NO: 7 was identified using standard methods.

Detail Description Paragraph - DETX (154):

[0191] Cloning and Sequencing of the Talaromyces Emersonii Glucoamylase Gene

Detail Description Paragraph - DETX (208):

[0242] Expression of Talaromyces Emersonii Glucoamylase in Yeast

Detail Description Paragraph - DETX (211):

[0245] The yeast cells were grown at 30.degree. C. for 3 days in Sc-ura medium followed by growth for 3 days in YPD. The culture was then centrifuged and the supernatant was used for the thermostability assay described in the "Materials and Method" section.

Detail Description Paragraph - DETX (216):

[0249] 200 ml culture broth from fermentation of *A. niger* HowB112 harboring the Talaromyces emersonii gene was centrifuged at 9000 rpm and dialyzed against 20 mM NaOAc, pH 5 over night. The solution was then applied on a S Sepharose column (200 ml) previously equilibrated in 20 mM NaOAc, pH 5. The glucoamylase was collected in the effluent, and applied on a Q Sepharose column (50 ml) previously equilibrated in 20 mM NaOAc, pH 4.5. Unbound material was washed of the column and the glucoamylase was eluted using a linear gradient from 0-0.3 M NaCl in 20 mM NaOAc over 10 column volumes. Purity of the glucoamylase fraction was checked by SDS-PAGE and only one single band was seen. The molecular weight was again found to about 70 kdal as seen for the wild type glucoamylase. The specific activity towards maltose was measured and a specific activity of 8.0 AGU/mg (37.degree. C.) and 21.0 AGU/mg (60.degree. C.) were found which is in accordance the data on the wild type enzyme.

Detail Description Paragraph - DETX (221):

[0252] The saccharification performance of the Talaromyces emersonii glucoamylase was tested at different temperatures with and without the addition of acid .alpha.-amylase and pullulanase. Saccharification was run under the following conditions:

Detail Description Paragraph - DETX (226):

[0257] Recombinant Talaromyces emersonii glucoamylase produced in *A. niger* 0.24 or 0.32 AGU/g DS

Detail Description Paragraph - DETX (236):

[0266] The thermal stability of recombinant Talaromyces emersonii glucoamylase expressed in yeast (purified using the method described in Example 9) was determined at 70.degree. C., pH 4.5, 0.2 AGU/ml using the method described above in the "Material and Methods" section as "Thermal Stability I (T.sub.1/2 (half-life) determination of AMG".

Detail Description Paragraph - DETX (237):

[0267] FIG. 13 shows the result of the test. The T/sub.1/2 of the recombinant Talaromyces emersonii glucoamylase expressed in yeast was determined to about 110 minutes at 70.degree. C.

Claims Text - CLTX (6):

6. The process of claim 1, wherein said glucoamylase is derived from Talaromyces emersonii.

PGPUB-DOCUMENT-NUMBER: 20040115779

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040115779 A1

TITLE: Fermentation process

PUBLICATION-DATE: June 17, 2004

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APPL-NO: 10/ 472256

DATE FILED: September 19, 2003

PCT-DATA:

APPL-NO: PCT/DK02/00179

DATE-FILED: Mar 19, 2002

PUB-NO:

PUB-DATE:

371-DATE:

102(E)-DATE:

US-CL-CURRENT: 435/105, 435/252.31 , 435/254.3

ABSTRACT:

The present invention relates to an improved process for producing a fermentation product.

----- KWIC -----

Detail Description Paragraph - DETX (85):

[0090] Other contemplated *Aspergillus* glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct. 12-17, 1997, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an *Aspergillus awamori* glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus, Talaromyces duponti (U.S. Pat. No. 32,153), Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus *Clostridium*, in particular *C. thermoamylolyticum* (EP 135,138), and *C. thermohydrosulfuricum* (WO 86/01831).

Claims Text - CLTX (4):

4. The process of claims 1-3, wherein the carbohydrate-source generating enzyme is a glucoamylase, in particular derived from *Aspergillus niger* or *Talaromyces emersonli*; or beta-amylase, in particular derived from barley; or a maltogenic amylase, in particular derived from *Bacillus stearothermophilus*.

PGPUB-DOCUMENT-NUMBER: 20040096952

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040096952 A1

TITLE: Alpha-amylase variant with altered properties

PUBLICATION-DATE: May 20, 2004

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APPL-NO: 10/ 477725

DATE FILED: November 14, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2001 00760	2001DK-PA 2001 00760	May 15, 2001
DK	PA 2001 00981	2001DK-PA 2001 00981	June 22, 2001
DK	PA 2001 00982	2001DK-PA 2001 00982	June 22, 2001
DK	PA 2001 00998	2001DK-PA 2001 00998	June 26, 2001
DK	PA 2001 00999	2001DK-PA 2001 00999	June 26, 2001
DK	PA 2001 01443	2001DK-PA 2001 01443	October 2, 2001

PCT-DATA:

APPL-NO: PCT/DK02/00319

DATE-FILED: May 15, 2002

PUB-NO:

PUB-DATE:

371-DATE:

102(E)-DATE:

US-CL-CURRENT: 435/202, 435/252.31 , 510/226 , 510/320

ABSTRACT:

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered properties relative to the parent alpha-amylase.

----- KWIC -----

Detail Description Paragraph - DETX (2271):

[2319] In another embodiment the composition comprises beside a variant of the invention a glucoamylase, in particular a glucoamylase originating from *Aspergillus niger* (e.g., the G1 or G2 A. niger AMG disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from *Aspergillus niger* are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102, or a variant therefore, in particular a variant disclosed in WO 00/04136 or WO 01/04273 or the Talaromyces emersonii AMG disclosed in WO 99/28448.





PGPUB-DOCUMENT-NUMBER: 20030187243

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030187243 A1

TITLE: Novel expression-regulating sequences and expression products in the field of filamentous fungi

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 257629

DATE FILED: April 11, 2003

PCT-DATA:

APPL-NO: PCT/NL01/00301

DATE-FILED: Apr 17, 2001

PUB-NO:

PUB-DATE:

371-DATE:

102(E)-DATE:

US-CL-CURRENT: 536/23.2, 435/200 , 435/252.3 , 435/320.1 , 435/6 , 435/69.1 , 435/72

ABSTRACT:

The invention pertains to novel proteins corresponding to Chrysosporium glycosyl hydrolases of families 7 and 10, exhibiting a minimum amino acid identity of 70 and 75%, respectively, with the amino acid sequence of SEQ ID No's 2 and 4, and to a protein corresponding to a Chrysosporium glyceraldehyde phosphate dehydrogenase, exhibiting at least 86% amino acid identity with the partial amino acid sequence of SEQ ID No. 6. The invention further relates to nucleic acid sequences encoding these proteins, and especially to promoter sequences regulating the expression of the corresponding genes. The preferred host for expressing these genes is a fungus, especially a Chrysosporium strain.

----- KWIC -----

Summary of Invention Paragraph - BSTX (40):

[0037] An expression-regulating region is a DNA sequence recognised by the host Chrysosporium strain for expression. It comprises a promoter sequence operably linked to a nucleic acid sequence encoding the polypeptide to be expressed. The promoter is linked such that the positioning vis-a-vis the initiation codon of the sequence to be expressed allows expression. The promoter sequence can be constitutive or inducible. Any expression regulating sequence or combination thereof capable of permitting expression of a polypeptide from a Chrysosporium strain is envisaged. The expression

regulating sequence is suitably a fungal expression-regulating region e.g. an ascomycete regulating region. Suitably the fungal expression regulating region is a regulating region from any of the following genera of fungi: Aspergillus, Trichoderma, Chrysosporium, Hansenula, Mucor, Pichia, Neurospora, Tolypocladium, Rhizomucor, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea e.g. the cellobiohydrolase promoter from Trichoderma, glucoamylase promoter from Aspergillus, glyceraldehyde phosphate dehydrogenase promoter from Aspergillus, alcohol dehydrogenase A and alcohol dehydrogenase R promoter of Aspergillus, TAKA amylase promoter from Aspergillus, phosphoglycerate and cross-pathway control promoters of Neurospora, aspartic proteinase promoter of Rhizomucor miehei, lipase promoter of Rhizomucor miehei and beta-galactosidase promoter of Penicillium canescens. An expression regulating sequence from the same genus as the host strain is extremely suitable, as it is most likely to be specifically adapted to the specific host. Thus preferably the expression regulating sequence is one from a Chrysosporium strain.

Summary of Invention Paragraph - BSTX (50):

[0047] Suitable examples of signal sequences can be derived from yeasts in general or any of the following specific genera of fungi: Aspergillus, Trichoderma, Chrysosporium, Pichia, Neurospora, Rhizomucor, Hansenula, Humicola, Mucor, Tolypocladium, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea. Signal sequences that are particularly useful are often natively associated with the following proteins a cellobiohydrolase, an endoglucanase, a beta-galactosidase, a xylanase, a pectinase, an esterase, a hydrophobin, a protease or an amylase. Examples include amylase or glucoamylase of Aspergillus or Humicola (4), TAKA amylase of Aspergillus oryzae, alpha-amylase of Aspergillus niger, carboxyl peptidase of Mucor (U.S. Pat. No. 5,578,463), a lipase or proteinase from Rhizomucor miehei, cellobiohydrolase of Trichoderma (5), beta-galactosidase of Penicillium canescens and alpha mating factor of Saccharomyces.